Influence of the Chlamydia psittaci Type III Secretion System on the innate immune response of chicken macrophages

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The Type III secretion system (T3SS) of *Chlamydiaceae* plays an important role at different stages of their biphasic developmental cycle like for instance i) during entry, when inducing actin recruitment to the entry site following translocation of the T3SS effector protein tarp, ii) during resistance to phagolysosomal fusion through modification of the inclusion membrane and iii) at the end of the developmental cycle when reticulate bodies detach from the inclusion membrane and differentiate to elementary bodies. The T3SS is highly conserved among several G^- bacteria and plays also a role in regulating the innate immune response of the host cell following infection with pathogens such as *Shigella* spp., *Pseudomonas* spp. and *Burkholderia* spp. as well as *Chlamydia trachomatis*.

Chlamydia psittaci also possesses a functional T3SS. Primary replication takes place in epithelial cells in upper respiratory tract. Later on, the bacteria can be found in epithelial cells and macrophages of the lower respiratory tract. Subsequently, *C. psittaci* can be found in plasma and blood monocytes, resulting in a systemic infection. Unfortunately, less is known about the underlying host innate immune response of *C. psittaci* infected macrophages and monocytes. As monocytes/macrophages play such an important role in the innate immune system, it is rather unique that *C. psittaci* as well as other *Chlamydiaceae* are able to survive and even replicate within those cells. In this way, the hypothesis arose that the T3SS might play a role in this process.

To investigate if *C. psittaci* T3SS plays a significant role in regulating innate immune response, HD11 chicken monocytes/macrophages, a well established "*in vitro*" model for studying bacterial host cell interactions were used. We determined the cytokine response following *C. psittaci* infection of HD11 cells by examining gene transcripts of IL-1β, Caspase 1, TNF-α, IL-6, MIF, IL-3, IL-10, IL-12p35, GM-CSF, chemokines (CXCLi2, CXCLi1, CCLi3 and IL-16) and toll like receptors (TLR2, TLR3, TLR4, TLR5, TLR7, TLR21) at different time points (2h, 4h, 8h, 12h and 18h) during an infection with the virulent *C. psittaci* strain 92/1293. Experiments were conducted in the presence and absence of the Type III secretion inhibitor INP0007. The results indicate that, dependent on the stage of the developmental cycle of *C. psittaci*, the T3SS has an influence on the host pro-inflammatory cytokine gene expression level (IL-1β, Caspase 1, TNF-α, MIF, IL-6 and IL12-p35), on the pro-inflammatory chemokine gene expression level (CXCLi2, CXCLi1 and CCLi3), on the growth factor GM-CSF gene expression level, on the expression level of the activation marker iNOS and on the NO production by HD11 cells. Furthermore, the T3SS do not regulate the anti-inflammatory response regarding to IL-10 and also the expression level of the pro-inflammatory chemokine IL-16. Interestingly, looking at the expression level of the toll like receptors, TLR21, which is a unique intracellular avian Toll like receptor for chickens with a broad DNA ligand specificity, is the most upregulated TLR. This TLR gene expression is also regulated by the T3SS at late time points in the infection.